

Open Literature Review Summary

Chemical Name: Imidacloprid

PC Code: 129099

ECOTOX Record Number and Citation:

MRID Number: 47800517

Decourtye, A., J. Devillers, S. Cluzeau, M. Charreton, and M.H. Pham-Delegue. 2004. Effects of imidacloprid and deltamethrin on associative learning in honeybees under semi-field and laboratory conditions. *Ecotoxicology and Environmental Safety*. 57: 410-419.

Purpose of Review (DP Barcode or Litigation): N/A

Date of Review: 03/24/12

Summary of Study Findings:

Summary: This study evaluated the sub-lethal effects of imidacloprid and deltamethrin on honeybees in both semi-field and laboratory conditions. A sugar solution containing 24 µg/kg of imidacloprid or 500 µg/kg of deltamethrin was offered to a colony in an outdoor flight cage. Deltamethrin had a lethal effect on worker bees, imidacloprid did not. The contaminated syrup with imidacloprid and deltamethrin induced a decrease in both foraging activity on the food source and activity at the hive entrance. Free flying foragers were used for the proboscis extension reflex under laboratory conditions. No impact of deltamethrin was found on learning performance, while significant effects were found with imidacloprid in both semi-field and laboratory conditions.

Methods: The study used colonies of Italian honey bees (*Apis mellifera ligustica* L.) with about 4000 workers and a fertile one year-old queen. The honey bees were confined in a 10-comb Dadant hive with 3 combs (one brood comb, one honeycomb, and one empty comb). The honeybees were obtained from a beekeeping company (Pasini, Italy), and had received no chemical treatments for at least 4 weeks prior to experiments. The colonies were maintained in an outdoor flight cage (2.5m x 2.5m, 2m high) covered with an insect-proof cloth (2mm x 2mm mesh) and a ground covered with a double layer of clear polyethylene plastic. Bees were fed on a feeder positioned 1.5 m from the hive entrance, filled with sucrose solution (500g/kg, 1% acetone vol./vol.) and pollen. The sucrose solution and pollen were renewed daily except during weekends. The sucrose solution was provided in a glass bottle set upside down, covered with aluminum paper to avoid exposure to light. The pollen was offered in a sheltered plastic dish and was from commercial sources. This pollen was analyzed for background contamination to detect imidacloprid and its three metabolites (hydroxy-imidacloprid, olefin, and 6-chloronicotinic acid), and deltamethrin. According the analyses, the pollen was free of imidacloprid, its metabolites, and deltamethrin. The food solution and pollen were removed from the flight cage during each behavioral recording session.

Technical grade imidacloprid (98%) and deltamethrin (99%) were purchased from Cluzeau Info Labo, and they were dissolved in acetone to make stock solutions that were diluted to final concentrations of 24µg/kg of imidacloprid and 500 µg/kg of deltamethrin.

The final concentration of acetone was 1% (vol./vol.). The treatment solutions were prepared before each experiment and then stored up to 2 weeks at -18°C. Each day the food solutions were defrosted at ambient temperature and natural daylight before their use.

Flight cage experiments: The responses of honey bees before and after exposure to insecticide were compared on the same colonies. Therefore, actual control colonies were not used. Three feeding periods were applied:

1. 500g/kg sucrose solution (1% acetone vol./vol.) delivered in both the artificial flower feeding and a standard feeder placed in the cage out of the experimental period
2. Insecticide added 500g/kg sucrose solution
3. 500g/kg sucrose solution (1% acetone vol./vol.) again. Experiments were conducted in June-July (2000 and 2001).

The foraging activity and learning performances were evaluated using an artificial flower feeder. The feeder contained six feeding sites distributed on a circular gray tray (50 cm diameter). Each artificial flower was a plastic Petri dish containing glass balls and filled with a sucrose solution. The level of sucrose solution in the Petri dish was maintained as constant. On each side of the feeding sites, an odor could diffuse (pure linalool; 95-97% purity). To limit the influence of visual spatial cues, the artificial feeder rotated slowly (1/3 rpm). The device was placed 1.5m from the hive entrance.

To initiate the recruitment of foragers, approximately 100 workers were placed on the artificial feeder. The foragers, were conditioned to the linalool associated with the food solution in each of the six artificial flowers. Each bee visiting the device was tagged with a color dot on the thorax. The number of tagged bees on the artificial feeder was noted every 5-min as a measure of foraging activity. When the population of marked foragers was stabilized (about 200 individuals), they were then conditioned (pairing odor/sucrose reward) over one day from 14:00 to 16:00 hours GMT. Testing was carried out on the following days from 10:00 to 11:00h or 14:00 to 15:00h GMT depending on the meteorological conditions. The testing device was set with 3 scented sites alternating with 3 unscented sites, without any food reward. The device was presented for 5 min and then replaced by the conditioning device for 15 min, with the odor being again associated with a sucrose solution. For each observation (every 30s over the 5 min observation time), the visits on either the scented sites or the unscented ones were noted. After each test, the tray was cleaned with ethanol and the Petri dishes were changed. The volume of sucrose solution was measured. Air temperature in the cages fluctuated between 23°C and 35 °C for imidacloprid, and between 27 °C and 37 °C for deltamethrin. The sky was cloudy and wind speed slight throughout the two studies.

Any dead bees found on the ground were counted and discarded daily except on weekends. Brood area and food quantities were assessed, as well as anomalies in behavior and development. Throughout the experiment, a bee counter set at the hive entrance evaluated the activity of the colony by measuring the number of bees leaving and entering the hive as a function of time at a sampling interval of 15 minutes. At the end of the interval, the counter delivered the number of bees that had entered and left the colony during that interval.

Laboratory experiments – olfactory conditioning of PER: At the end of each experimental period in the outdoor flight cage, color-marked foraging bees were collected on the artificial flower and caged in groups of 30-50 individuals. They were maintained in an incubator at $25\pm 2^\circ\text{C}$, $40\pm 10\%$ RH, and in the dark. They were starved for 4hrs prior to odor conditioning in the PER assay. The bees in this experiment were conditioned based on the proboscis extension reflex and classical temporal pairing of a conditioned stimulus and an unconditioned stimulus. For more details of this PER assay, please see the study report.

Prior to conditioning, honeybees were selected for showing a proboscis extension reflex after stimulation of the antennae with a 300g/kg sucrose solution. Phenylacetaldehyde was chosen as the conditioned stimulus. Three conditioned trials were carried out at 20 min intervals on average (trials C1-C3). The conditioned proboscis extension was recorded as a yes or no response when the odor alone was delivered.

Statistical Analysis: In the flight cage experiments, the number of visits to the scented or unscented sites were compared with the hypothesized equal distribution (50% of foragers on either sites) by χ^2 test. In the conditioned PER tests, the number of reflex responses in the treated groups and in the control group (before or after imidacloprid treatment period) were compared.

Results: The treatment period with imidacloprid did not lead to additional mortality, whereas the number of dead bees found on the ground of the flight cage during the deltamethrin administration was about 2x greater than before and after this period (**Table 1**). In addition, trembling and paralysis were noted in honey bees laying on the ground in the deltamethrin exposure test. With three attempts, samples of bees affected by these symptoms were collected to see whether they would recover with time, but all of the bees died within 4hrs. A treatment-related difference was found in the syrup consumption rates (**Table 2**). The addition of either imidacloprid or deltamethrin induced a reduction in consumption by a factor of 3. The duration of this effect was only during the exposure period for deltamethrin but persisted through the next observation period with clean sucrose solution for imidacloprid.

Table 1
Mortality^a in relation to treatment

Chemicals	Before treatment	Treatment	After treatment
Imidacloprid	70.0 ± 16.4 ($n = 5$) ^b	57.7 ± 25.9 ($n = 4$)	83.4 ± 31.9 ($n = 3$)
Deltamethrin	74.9 ± 22.2 ($n = 4$)	156.1 ± 20.9 ($n = 4$)	88.0 ± 18.8 ($n = 4$)

^aData represent mean number of dead workers bees per day (\pm SEM) which were found on ground of flight cage.

^bNumber of days where mortality was recorded.

Table 2
Consumption^a of sucrose solution (mL) distributed by the artificial flower feeder

Chemicals	Before treatment	Treatment	After treatment
Imidacloprid	186.0 ± 39.3 ($n = 6$) ^b	57.9 ± 9.7 ($n = 5$)	38.2 ± 5.3 ($n = 5$)
Deltamethrin	93.2 ± 20.2 ($n = 5$)	30.7 ± 8.0 ($n = 4$)	74.0 ± 14.1 ($n = 4$)

^aData represent mean volume of syrup consumption per day (\pm SEM).

^bNumber of days where consumption was recorded.

There was a decrease in comb area containing capped brood between the beginning and the end of experiments with the two chemicals studies. The reduction of brood size was higher in the imidacloprid study (**Table 3**). The last control visit revealed irregular

capped brood area in both colonies. The honey and pollen stores in the colony exposed to imidacloprid were reduced at the end of the experimental period. The study authors state that deltamethrin had no impact on food stores, but the data in Table 3 show the absence of stored pollen. Thus it appears that deltamethrin may also impact food storage.

Table 3
Development of the colony over the time in relation to treatment with imidacloprid ($24 \mu\text{g kg}^{-1}$) and deltamethrin ($500 \mu\text{g kg}^{-1}$)

Parameters	Imidacloprid			Deltamethrin		
	Before treatment (21/06/00) ^a	Treatment (28/06/00)	After treatment (07/07/00)	Before treatment (18/06/01)	Treatment (02/07/01)	After treatment (09/07/01)
Comb area containing capped brood (cm^2)	850.5 ^b	966.8	534.0	1423.9	964.4	919.9
Eggs	+	+	+	+	+	+
Larvae	+	+	+	+	+	+
Uncapped honey	+	+	0	+	+	+
Capped honey	+	+	+	0 ^d	0	+
Pollen	+	+	0	+	0	0
Remarks			Irregular brood			Irregular brood

^a Date where control visit of colony was carried out.

^b Comb area were calculated with ellipse method (Fresnaye and Lensky, 1961).

^c Symbol indicates the presence of parameter considered.

^d Symbol indicates the absence of parameter considered.

Foraging activity on the artificial flower feeder showed that imidacloprid and deltamethrin had a similar repellent effect (**Figure 1A and B**). From the beginning of the feeding period with the two chemicals, there was a decrease in the number of foraging bees in comparison to the observation before the addition of spiked sucrose solutions. Low foraging activity was prolonged throughout the overall period of imidacloprid or deltamethrin application (i.e., 7 and 8 days, respectively). After the treatment with deltamethrin, the bees fed clean sucrose solution resulted in an increase in foraging bees at the feeder. However, low levels of foraging at the feeder remained in the observation period after imidacloprid spiked solution were replaced with clean sucrose solution.

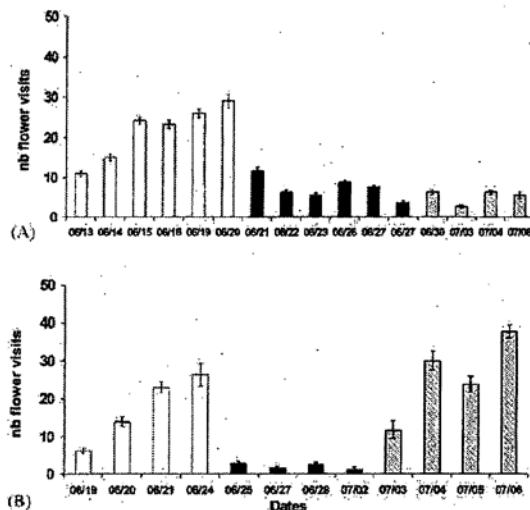


Fig. 1. Foraging activity of honeybees on artificial flower feeder in relation to treatment with imidacloprid (A), and deltamethrin (B). Bars give the mean (\pm SEM) number of foraging bees which were recorded on the feeding sites. White bars: control sucrose solution before treatment period; black bars: imidacloprid-added sucrose solution ($24 \mu\text{g kg}^{-1}$) or deltamethrin-added sucrose solution ($500 \mu\text{g kg}^{-1}$); gray bars: control sucrose solution after treatment period.

Before the treatments, the number of visits to the odor sites (linalool) were significantly greater than visits to unscented sites (94-96% of landings on scented sites) (**Figure 2**). With replacement of the clean sucrose solution by imidacloprid-spiked sucrose, the percentage of foragers visiting the scented sites was reduced (60% of landings on scented sites). Despite this inability to discriminate olfactory stimuli, the number of landings on scented sites was significantly higher than a randomized distribution between scented and unscented sites. Going back to the control solution after the treatment period, the foragers showed a high level of olfactory discrimination performance (90% of landings on scented sites). In the deltamethrin study, the sites scented with linalool were discriminated (82-96% of landings on scented sites) from unscented sites during all experimental periods.

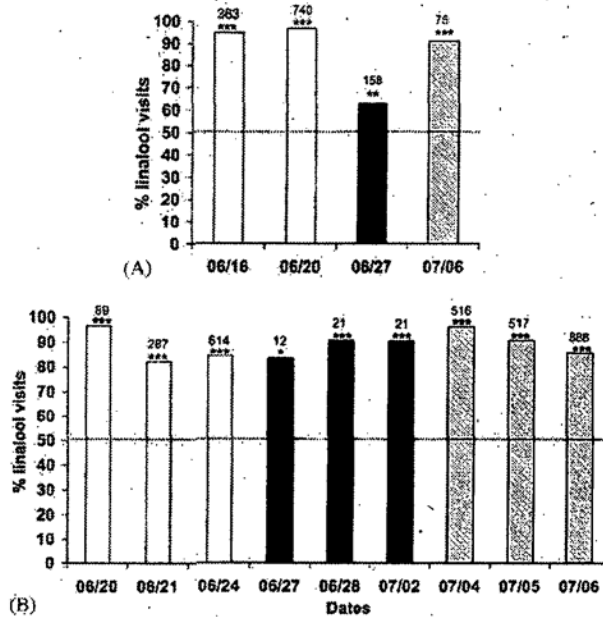


Fig. 2. Olfactory learning performance of free-flying foragers in relation to treatment with imidacloprid (A), and deltamethrin (B). Bars give the percentage of foragers visiting scented sites of the artificial flower feeder. White bars: control sucrose solution before treatment period; black bars: imidacloprid-added sucrose solution ($24 \mu\text{g kg}^{-1}$) or deltamethrin-added sucrose solution ($500 \mu\text{g kg}^{-1}$); gray bars: control sucrose solution after treatment period. The total number of foragers is indicated above the bars. The observed numbers of visits were compared to hypothesized equal distribution of landings on the scented sites and unscented sites, shown as the 50% line (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Flight activity was greater before exposure to imidacloprid than during the exposure period (**Figure 3**). With deltamethrin exposure, there were no changes in the activity at the hive entrance noted by the counter.

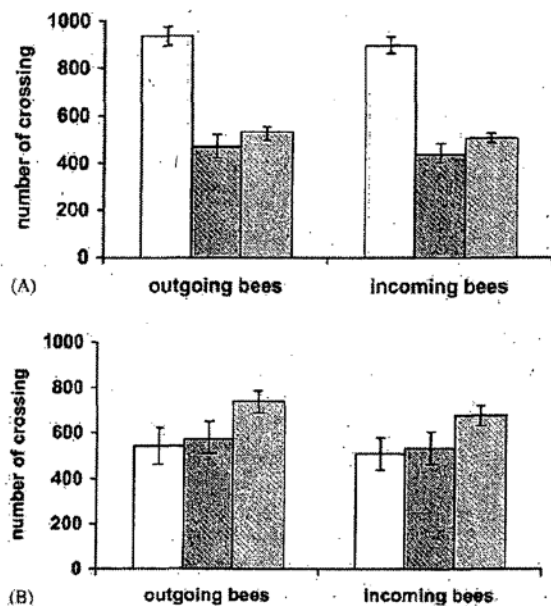


Fig. 3. Flight activity of the colony registered by bee counter in relation to treatment with imidacloprid (A), and deltamethrin (B). Bars give the mean (\pm SEM) number of crossing which were recorded by the counter (1 h per day for testing period and 2 h per day for conditioning period). White bars: control sucrose solution before treatment period; black bars: imidacloprid-added sucrose solution ($24 \mu\text{g kg}^{-1}$) or deltamethrin-added sucrose solution ($500 \mu\text{g kg}^{-1}$); gray bars: control sucrose solution after treatment period.

In the PER assay, the number of conditioned responses differed according to the feeding period in the flight cage (**Figure 4**). The PER response decreased in the period with imidacloprid exposure relative to the period prior to exposure. The reduction in olfactory learning performance was also noted in foraging bees collected 9 days after the end of the imidacloprid treatment (trials C2 and C3). In contrast, the foragers fed deltamethrin-added solution had responses equivalent to foragers fed clean solution. In addition, when comparing the motor reflex responses obtained when antennae were contacted with a sucrose solution, only the foraging bees collected after removal of the imidacloprid spiked solution showed a significant decrease in PER rates (52-58%) relative to responses recorded before and during treatment (90-100%). For deltamethrin, the foraging bees collected during the three experimental periods presented similar PER rates (76-95% of reflex responses).

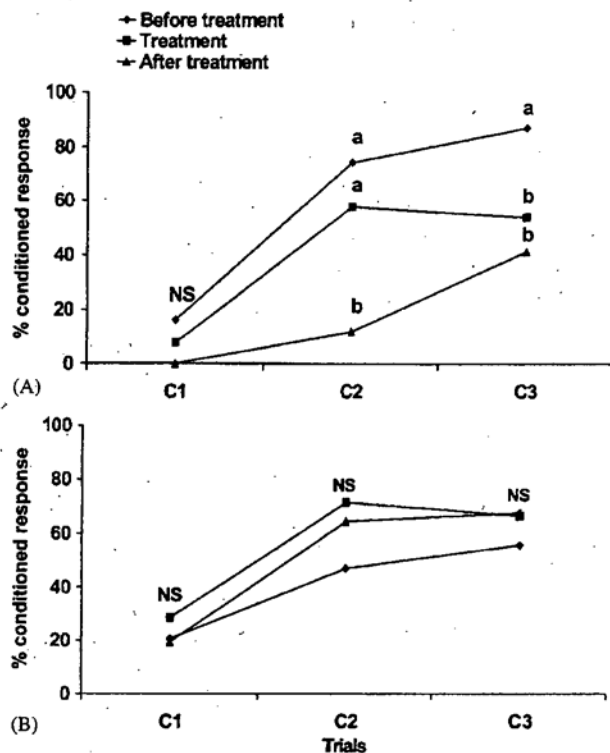


Fig. 4. Olfactory learning performance of restrained foragers bees during olfactory conditioning of PER in relation to treatment with imidacloprid (A), and deltamethrin (B). Number of bees per treatment group: 17–31 in imidacloprid study, 21–34 in deltamethrin study. The number of conditioned responses obtained during the conditioning trials (C2–C3, C1 showing the level of spontaneous response) was compared among experimental period using χ^2 test (2 df, $P < 0.05$; NS: non-significant), followed by multiple two-by-two comparisons using χ^2 test or Fisher's exact method (1 df). Different letters indicate significant differences in this test (corrected significance threshold $\alpha' = 0.016$).

Description of Use in Document (QUAL, QUAN, INV):

QUALITATIVE

Rationale for Use: This study presents useful information on sublethal and lethal effects associated with exposure to imidacloprid and deltamethrin. This information can be used to characterize the risk to bees from the exposure to imidacloprid or deltamethrin, and highlights a different mode of action related to each chemical. The study indicates a decrease in comb area containing capped brood between the beginning and the end of experiments with the two chemicals studied. In addition, the last control visit revealed irregular capped brood area in both colonies. This suggest the possibility of impacts on the brood-rearing capabilities of honey bees associated with both of these compounds, though there is uncertainty in these endpoints as it is possible that the enclosures may have impacted these endpoints (see limitations section below). The food storage in the imidacloprid exposed colony was also impacted, whereas deltamethrin did not appear to impact honey stores. Pollen storage was also absent after exposure to deltamethrin. Imidacloprid alone impacted learning performance and reflex responses, whereas

foragers performed similarly regardless of the presence or absence of deltamethrin in the sucrose during each observation period. These data suggest that both chemicals may impact food storage where the mechanistic cause may be associated with a decrease in foraging activity and performance at the sublethal level for imidacloprid and a decrease in the number of foragers due to lethality for deltamethrin, thereby leading to potential impacts to brood production and/or survival.

Limitations of Study: The study report states that marked bees were collected on the artificial flower feeder and used for the laboratory experiments. The data do not indicate the extent to which the bees fed on the sucrose solution in the artificial flower. Therefore, this lack of data on individual consumption represents an uncertainty. The lack of the use of a negative control, or any other control for that matter also introduces uncertainty into the study results. While the same colony was used across observation periods, the climate or other environmental factors may change over the course of the experiment and affect the outcome of the study results. Without the use of control colonies at each time point, this uncertainty cannot be evaluated. In addition, the enclosures themselves may pose a stress to honey bee colonies. Some of the differences in food stores or capped brood may be a function of stress from the enclosures in which the colonies were placed. No control colonies were used, and so this potential stress of the enclosures could not be evaluated. The study does not provide quantitative measures of food storage. The study report does not provide raw data to confirm the statistical conclusions.

Primary Reviewer:

Joseph DeCant, Ecologist, ERB5